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FOREWORD

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J. Cao

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TECHNICAL RESEARCH PROGRESS REPORT

Principal Investigator: Jian Cao, M.D.
Mentor: Stanley Zucker, M.D.
Grant Number: DAMD17-98-1-8160
Project Title: Examination of the role of membrane type-1 matrix metalloproteinase (MT1-MMP) in breast cancer metastasis

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MT-MMPs are able to activate progelatinase A on the surface of tumor cells by producing an initial cleavage in the N-terminal propeptide domain followed by an autolytic cleavage. Because of its central role in cell surface proteolysis, a more complete understanding of the role of MT-MMPs in cancer will expedite progress in therapy of metastasis. To this end, this project has been focused on the role of MT1-MMP in experimental breast cancer invasion and metastasis.

Task 1. Examination of the role of wild type membrane type-1 matrix metalloproteinase (MT1-MMP) in breast cancer metastasis

To directly address the role of MT1-MMP in experimental breast cancer, human breast cancer cell line, MDA-MB 436 that does not express MT1-MMP (confirmed by Northern blot), was used in this project. Green fluorescent protein (GFP) was employed as a tumor cell marker at metastatic sites and also as a visible fluorescent tag to localize MT1-MMP fusion molecules within cells. Since a deletion mutant of MT1-MMP lacking the C-terminal cytoplamic tail of MT1-MMP does not lose the function in terms of progelatinaes A activation, GFP tag was fused into the C-terminus of MT1-MMP. By gelatin zymography, MT1-MMP/GFP chimera was able to cleave progelatinase A and further induced gelatinase A activation. Alternatively, the distribution of MT1-MMP\GFP was examined in transiently transfected MDA-MB-436 cells. GFP fluorescence exhibited diffuse distribution in transfected cells. In contrast, MT1-MMP\GFP accumulated primarily in the endoplasmic reticulum and in the perinuclear golgi apparatus of the transfected cells. Fluorescence on the leading edge of plasma membrane was also observed but seems limited in vitro. By immunofluorescent study using MT1-MMP antibody and TRITC conjugated anti-mouse IgG, the same profiles of MT1-MMP transfected cells were noted. These data indicated that MT1-MMP primarily accumulated in the perinuclear region of transfected cells and may require a signaling mechanism to facilitate membrane trafficking; GFP does not change the distribution of fused protein.

To better understanding the role of MT-MMP in human breast cancer metastasis, a stable cell line expressing MT-MMP was established. MDA-MB-436 cells were transfected with MT1-MMP plasmids by calcium

phosphate method and stable clones survived in G418 conditioned media were selected by fluorescent microscopy. Fluorescent clones were further examined by gelatin substrate zymography. The clone expressing MT1-MMP/GFP chimera protein and inducing progelatinase A activation was expanded for further experimentation.

We previously showed that MT1-MMP transfected COS-1 cells increased the invasive ability more than two-fold compared with vector trasfected cells. Here, I used GFP as a tag molecule to trace the movement of human breast cancer cells expressing MT1-MMP.

Native MDA-MB-436 cells produce slow growing, poorly invasive tumors. Injection of MDA-MB-436 cells stably transfected with MT1-MMP/GFP cDNA into the inferior mammary fat pad of female nude mice resulted in enhancement of tumor growth and local metastasis as compared to GFP-alone transfected tumor cells. Green fluorescent protein was employed to monitor metastasis. MT1-MMP/GFP transfected tumor lysates demonstrated much high levels of active progelatinase A as compared to GFP-transfected tumor lysates.

Task 2. Determine whether soluble forms of MT1-MMP play a role in experiment breast cancer dissemination (months 18-30).

As initially planned, this task will be performed in year 2.

Task 3. Investigate intracellular trafficking and localization of MT1-MMP in transfected breast cancer cells (months 25-36).

(A) Thrombin, concanavalin A, and PMA may upregulate MT1-MMP translocation to the plasma membrane.

Employing surface biotinylation, immunoprecipitation, and ^{125}I-TIMP-2 binding to characterize the rapid appearance of MT1-MMP on the cell surface, we demonstrated that concanavalin A-induced MT1-MMP expression and activation of MMP-2 in HT1080 sarcoma cells occurs within 3 hours. Concanavalin A -induced trafficking of MT1-MMP from the trans Golgi network(TGN)/endosomal compartment to the cell surface occurs within 5-20 minutes. Trafficking was inhibited by Brefeldin A, an inhibitor of the Golgi apparatus, but not by cycloheximide, an inhibitor of protein synthesis. Similar results were also noted in PMA-stimulated HT1080 cells. The intracellular storage compartment for MT1-MMP and the physiologic control of trafficking remain to be characterized (manuscript in preparation).

(B) Pro-sequence of MT1-MMP serves as an intramolecular chaperone for the production of active MT1-MMP in transfected COS-1 cells.

The goal of this study is to further clarify the role of the propeptide domain of MT1-MMP in trafficking of MT-MMP to the plasma membrane and in maintaining the function of the plasma membrane-inserted enzyme. We propose that the propeptide sequence of MT1-MMP serves as an

intramolecular chaperone in protein folding. To explore the concept, we have co-transfected COS-1 cells with the plasmids encoding the N-terminal propeptide domain of MT1-MMP and the MT1-MMP cDNA lacking the entire propeptide sequence and have demonstrated reconstitution of function of MT1-MMP.

A cDNA encoding the N-terminal propeptide sequence of MT1-MMP (MT1-109) and cDNA encoding propeptide deleted MT1-MMP (MTΔpro) were inserted in expression vectors which were then transfected into MT-MMP deficient COS-1 cells. Co-transfection of COS-1 cells with both expression vectors resulted in reconstitution of MT1-MMP function in terms of facilitating ^{125}I labeled TIMP-2 binding to transfected COS-1 cells and subsequent activation of progelatinase A. Transfection of cells with either cDNA alone or cDNA for the propeptide of collagenase-1 with MTΔpro resulted in non-functional cells. Smaller cDNA mutations of the open reading frame of the N-terminal propeptide of MT1-MMP were employed to delineate critical conserved regions of the molecule required as a membrane-bound enzyme. These data indicated that the MT1-MMP prosequence acts as an intramolecualr chaperone and is necessary for the correct folding of the MT1-MMP *in vivo* (manuscript in preparation).

Task 4. This postdoctoral fellowship grant provides me with important experience in studying experimental animal models of breast cancer and in evaluating the effect of mutating MT1-MMP cDNA on the frequency of experimental metastasis. Training in Dr. Stanley Zucker's lab, has resulted in development of my molecular techniques. Also, I learned to perform experiments on animals. More importantly, Dr. Zucker meets me for 2 and a half hours per week to discuss experimental plans and to analyze the results from completed experiments. This experience gave me the opportunity to learn how to become an independent investigator using molecular biology techniques to study the role of MMPs in tumor invasion and metastasis.